PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of Jevnikar et al.
Serial No. 08/617,874
Filed: May 21, 1996
For: METHODS AND PRODUCTS FOR CONTROLLING IMMUNE RESPONSES IN MAMMALS

Examiner: M. Lubet
Group Art Unit: 1644
Date: June 1, 2000

SUPPLEMENTAL DECLARATION OF ANTHONY M. JEVNIKAR

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Anthony M. Jevnikar, do hereby declare and say as follows:

1. I am the co-inventor named in the above-identified application and I make this Declaration to supplement the information provided in the Declaration of Anthony Jevnikar dated September 27, 1999, and filed in this case on October 1, 1999 (hereafter "the first Jevnikar Declaration").

2. I have read and understood the Office Action mailed on December 2, 1999.

3. The Examiner has rejected claims 19 – 21, 28, 31 – 36 and 53 – 55 under 35 U.S.C. §103(a), as unpatentable over Lam et al. (U.S.P. 5,484,719), in view of Tobin et al., (U.S.P. 5,475,086) or Lernmark et al. (U.S.P. 5,792,620). The Examiner has also rejected claims 19 – 21, 28, 31 – 36, 42 and 53 – 55 as unpatentable over Lam et al. in view of Tobin et al. or Lernmark et al. in view of Carrington et al. (J. Virol, v. 64, p. 1590 (1990)). The Examiner has furthermore rejected claims 1, 5 – 10, 19 –

21, 28, 31 –36 and 53 – 55 under 35 U.S.C. § 103 (a), as unpatentable over Lernmark et al., Tobin et al., Weiner et al., (U.S.P. 5,643,868) and Zhang et al. (PNAS, v. 88, p. 10253 (1991)) in view of Lam et al. The Examiner has also rejected claims 1, 5 to 10, 19 – 21, 28, 31 – 36, 41 – 42 and 53 – 55 under 35 U.S.C. 103 (a), as unpatentable over Lernmark et al., Tobin et al., Weiner et al., Zhang et al., and Lamont et al. (Immunology, v. 66, p. 595 (1989)), in view of Lam et al. and further in view of Carrington et al.

4. My appreciation of the teachings of Lam et al. and Tobin et al. were set out in paragraphs 4 to 10 of the first Jevnikar Declaration and my appreciation of Weiner et al. and Zhang et al. in paragraphs 11 – 12 of that Declaration.

5. Lernmark et al. discloses the cloning and expression of a DNA sequence encoding human islet cell GAD, and discusses the preparation and purification of recombinant GAD. Lernmark et al. also suggests that purified recombinant GAD can be used, either by parenteral injection or orally, to induce immunological tolerance to GAD autoantigen in patients predisposed to or already mounting an immune response to GAD.

Lernmark et al., however, clearly did not conceive of producing GAD recombinantly in transgenic plants and administering plant material containing expressed GAD orally to produce immune tolerance. Lernmark et al. teaches only mass culture production of recombinant GAD, followed by purification of the protein before administration. The entire discussion in this patent is within the context of in vitro recombinant expression, as evidenced, for example, by the following comments: "Host cells containing DNA constructs of the present invention are then cultured to produce the human islet cell GAD polypeptides. The cells are cultured according to standard methods in a culture medium... (column 8, lines 32 – 34); "selection of a medium appropriate for the particular cell line used is within the level of ordinary skill in the art" (column 8, lines 46 – 47); and "the human islet cell GAD produced according to the present invention may be purified by affinity

chromatography" (column 8, lines 59 – 60). Within this context there is provided, in the passage referred to by the Examiner, a "shopping list" of suitable host cell types, including plant cells, for in vitro expression.

It is stated at column 2, lines 64 to 67, that "For large scale production the expressed human islet cell GAD polypeptides can be isolated from the cells by, for example, immunoaffinity purification".

There is nothing in this reference to suggest the expression of an antigen such as GAD in a transgenic plant, followed by oral administration of plant material containing the expressed antigen to a subject.

6. Lamont et al. discloses the oral administration of ovalbumin to suppress a systemic immune response to that antigen.

There is nothing in this reference to suggest the expression of an antigen such as GAD in a transgenic plant, followed by oral administration of plant material containing the expressed antigen to a subject.

7. The Examiner notes in the Office Action of December 2, 1999, that she has considered the first Jevnikar Declaration and states that "Dr. Jevnikar alleges that GAD produced in plant materials had an unexpected property with respect to GAD produced recombinantly in E.coli, since a plant extract from a transgenic tobacco plant expressing GAD induces a greater in vitro proliferative response in T cells from animals immunized with GAD than does purified GAD obtained by purifying GAD recombinantly expressed in E.coli". The Examiner indicates that Exhibit 2 of the first Jevnikar Declaration lacks (1) data comparing purified GAD obtained by expression in E. coli with purified GAD obtained by expression in plants and (2) further lacks data comparing the T cell response to plant material (extract) containing expressed GAD with the T cell response to a control plant material (extract) from a transgenic plant not expressing GAD.

8. Firstly, with respect to point 1, the first Jevnikar Declaration asserts, at paragraph 14, that "the inventors have found unexpectedly that plant material containing plant-expressed mouse GAD protein stimulated a greater proliferative response of GAD-primed T cells than highly purified recombinant mouse GAD expressed in E. coli" (emphasis added). Furthermore, the claims are also directed to use of a plant material containing a plant-expressed antigen such as GAD. The first Declaration did not compare purified GAD obtained by plant expression with purified GAD obtained by E. coli expression; hence, no such comparative data are provided.

9. With respect to point 2, concerning data comparing T cell responses to plant material containing expressed GAD with T cell responses to control plant material from a transgenic plant not expressing GAD, the attached Exhibits 1 and 2 provide such data, as will be explained in more detail below.

10. Exhibit 1 is a summary of studies published by the inventors in Nature Medicine, v. 3, pp. 793 – 796 (1997), in a paper entitled "Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance", a copy of which was filed with the applicant's response dated January 5, 1998.

11. In accordance with the protocol set out in Exhibit 1, NOD mice were treated orally either with plant material (potato slices or tobacco leaves) containing expressed mouse GAD or with control plant material obtained from empty vector-transformed plants (potatoes or tobacco). After challenge with purified recombinant mouse GAD by foot pad injection, spleen cells were removed and examined for their in vitro proliferative response to purified recombinant mouse GAD (pur. rGAD) or a non-relevant antigen (ovalbumin). As seen in the RESULTS section of Exhibit 1, when animals were treated orally with control plant material from empty-vector transformed plants (PM), their spleen cells showed proliferation in response to purified mouse GAD but not to ovalbumin, indicating an immune response to GAD.

This immune response to GAD was suppressed in animals treated orally with plant material containing expressed mouse GAD (PM + GAD), whose spleen cells showed no proliferative response to purified mouse GAD. It was not possible to compare the effect of oral treatment with plant material containing expressed GAD with oral treatment with purified, E. coli-produced recombinant GAD in this experiment as we simply could not commit the resources required for the large scale fermentation and large scale purification required to make, in E. coli, and purify the levels of GAD (around 1mg/day/animal) required for oral administration to induce immune tolerance.

12. The studies described in Exhibit 1 show that plant material containing expressed GAD, administered orally, suppresses or reduces the immune response of a mammal to the autoantigen GAD; this effect is not seen after oral administration of control plant material from empty-vector transformed plants.

13. Exhibit 2 attached shows the results of an experiment similar to that described in Exhibit 2 of the first Jevnikar Declaration. For clarification, Figure 1 of attached Exhibit 2 (page 2/3) is in fact a linear scale Y axis version of the Figure previously presented with a logarithmic Y axis in the first Jevnikar Declaration. Figure 2 of attached Exhibit 2 (page 3/3) now shows control results obtained using a control plant material from plants which express an irrelevant control protein, MHC class II alpha chain protein (I-Ak). This control exposes the test animals to plant material in the absence of the antigen, GAD, to which they have been sensitized.

14. Both Figures 1 and 2 show that when mice are primed with plant material containing expressed GAD (PGAD), their T cells proliferate in response both to purified recombinant mouse GAD (MGAD) and to plant material containing expressed GAD (PGAD), but that the response to PGAD is much greater than the response to MGAD. Figure 2 shows that, in contrast, animals primed with plant material from a transgenic plant not expressing GAD (I-Ak) showed no response to

MGAD. These animals did, however, show a proliferative response to the plant material containing expressed GAD (PGAD); since these animals are not sensitized to mouse GAD, as indicated by the lack of response to MGAD, this response to PGAD indicates the T cell response to plant proteins or other constituents other than the expressed mouse GAD.

15. These results indicate that a small fraction of the T cell response to PGAD challenge seen in PGAD-primed animals is likely due to plant material components other than the expressed mouse GAD. The greater magnitude of T cell response of PGAD-primed animals to PGAD challenge, whether compared with the response of the same animals to MGAD (as in the Figures 1 and 2 attached) or with the response seen in MGAD-primed animals challenged with MGAD (as seen in Figure 1 attached), indicates that there is an unexpected synergistic enhancement of T cell activation when GAD-reactive T cells are primed with plant material containing expressed GAD.

Although the absolute levels of T cell proliferation observed vary somewhat from experiment to experiment, the highest levels of proliferation were always seen in cells from PGAD-primed animals challenged with PGAD.

16. As indicated in the first Jevnikar Declaration, T cell activation is a prerequisite for the induction of immune tolerance, including oral tolerance. A T cell initially engages with an antigen when the antigen is presented to it by antigen presenting cells, leading to transmission of a first signal within the T cell. Depending on the presence or absence of further costimulatory signals, the T cell may progress to proliferation (immune response) or may become quiescent and unable to respond to the antigen (immune tolerance). Enhanced T cell activation, as seen after administration of plant material containing expressed GAD, can be expected to contribute to enhanced immune tolerance when subjects are treated orally with plant material containing expressed GAD, as described in this application.

17. Although the reason for the enhanced T cell activation seen after administration of plant material from transgenic plants containing expressed GAD is not presently known, it seems reasonable to postulate that other components of the plant material contribute to the observed synergistic effect. As noted at paragraph 16 of the first Jevnikar Declaration, lectins, which are common plant components, have been described as having immune-stimulating effects and may be contributing to the enhancement. It is interesting to note that plant lectins have been reported to induce secretion of IL-4 and IL-13 from human basophils and it is known that IL-4 is a key cytokine in the mediation of immune tolerance, through direction of the immune system towards a Th2 T cell response (Haas et al. (1999), Eur. J. Immunol., v. 29, pp. 918-927: copy enclosed).

18. The teachings of Lam et al., and Tobin et al., whether or not supplemented by the teachings of Lernmark et al. and/or Carrington et al., cannot, in my opinion, be combined to arrive at the present invention, as discussed above; neither the teachings of Wiener et al. and Zhang et al. regarding the use of a native mammalian autoantigen, nor the additional teaching of Lamont regarding oral administration of ovalbumin to produce immune tolerance, can supplement the teachings of Lam, Tobin, Lernmark and Carrington so as to arrive at an appreciation that plant material containing plant-expressed mammalian antigens could be administered orally to produce immune tolerance or that such material would produce enhanced T cell activation.

19. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false

statements may jeopardise the validity of the application or of any patent issued thereon.

Anthony M. Jevnikar

June 13, 2000
Date